Supporting Information

for

Programming Protein Patterns on DNA Nanostructures with Sequence Specific Polyamides

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Materials. Boc-β-Ala-PAM resin was purchased from Peptides International. Trifluoroacetic acid (TFA) was purchased from Halocarbon. Methylene Chloride (DCM) was obtained from Fisher Scientific and *N,N*-dimethylformamide (DMF) from EMD. EZ-Link TFP-PEO_3-Biotin was purchased from Pierce. Streptavidin was purchased from Rockland. All DNA oligonucleotides were purchased from Integrated DNA Technologies.

Polyamide Synthesis. Polyamide monomers were prepared as described previously.[1] Synthesis was performed using established protocols and all polyamides were characterized by MALDI-TOF and analytical HPLC.

1: (MALDI-TOF-MS) $[M+H]^+$ calc. for $C_{83}H_{117}N_{28}O_{17}S^+$ 1809.9, observed 1810.0

2: (MALDI-TOF-MS) $[M+H]^+$ calc. for $C_{83}H_{114}ClN_{26}O_{17}S_2^+$ 1846.8, observed 1846.5

3: (MALDI-TOF-MS) $[M+H]^+$ calc. for $C_{83}H_{117}N_{28}O_{17}S^+$ 1809.9, observed 1810.2

AFM Sample Preparation. The DX array was formed in two steps. Individual tiles **A**, **B**, **C**, and **D** were first annealed by mixing equimolar amounts of each of the four input strands at 1 μM concentration in TAEMg Buffer (40 mM Tris-HCl (pH 8.0), 20 mM acetic acid, 1 mM EDTA, 12.5 mM magnesium acetate). Each sample was heated to 95°C for 10 min and allowed to cool slowly over several hours to room temperature. The four tiles were then combined, heated to 45°C and allowed to cool to RT over approximately 12 hrs. For all of the experiments, polyamide was incubated with DX-**ABCD** (100 nM) for 1 hr prior to imaging. The polyamide concentration was 200 nM for **1** and **3**, and 150 nM for **2** which showed a propensity to bind at additional sites at higher concentrations. After the incubation, the sample was diluted in half which led to cleaner

AFM images. 5 µL of sample was spotted on freshly cleaved mica and allowed to absorb for 1 min. 2 µL of 1 µM Streptavidin was then added to the sample for 1 min. Imaging was then done using a DI Multimode Atomic Force Microscope. Calibration for the distance measurements was done by averaging four measurements of individual tiles in an untreated ABCD array. The reported spacings for each of the polyamides with streptavidin were calculated as the average distance between peaks in the graphs shown in Figures 3 and 4.

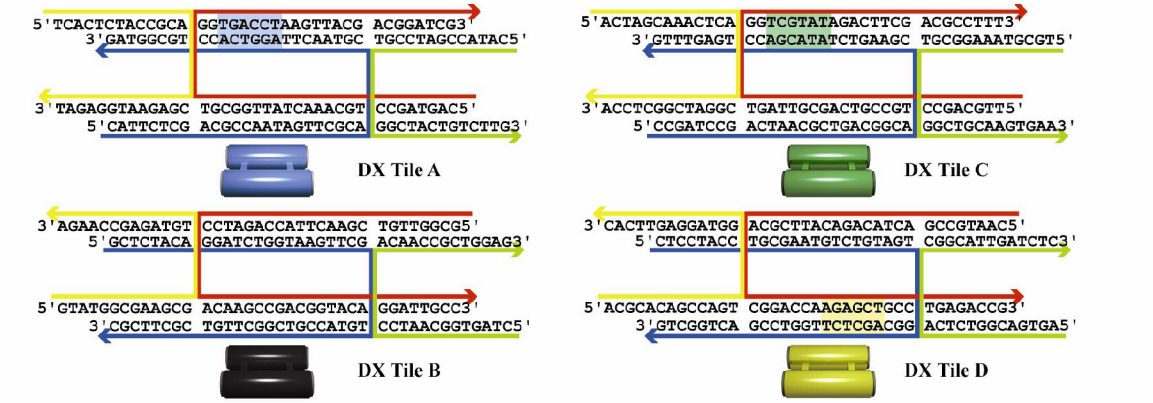

Figure S1. Full Schematics for each DX Tile. The colored highlights correspond to the polyamide binding site on each tile.

References:

(1) Baird, E. E.; Dervan, P. B., *J. Am. Chem. Soc.* **1996,** 118, 6141-6146.